Short- and long-term effects of conventional and artificial rearing strategies on the health and performance of growing lambs

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Short- and long-term effects of different rearing strategies on the health and performance of growing lambs

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Short title: Effects of artificial rearing of lambs

Abstract

Artificial rearing of young animals represents a challenge in modern ruminant production systems. This work aims to evaluate the short- and long-term effects of the type of rearing on the animal´s health, growth, feed utilization and carcass performance. Twenty-four pregnant ewes carrying triplets were used. Within each triplet set, lambs were randomly allocated to one experimental treatment: natural rearing on the ewe (NN); ewe colostrum for 24h followed by artificial rearing with milk replacer (NA); and 50g of colostrum alternative supplementation followed by artificial rearing (AA). Milk replacer, ryegrass hay and creep feed were offered *ad libitum* and each experimental group was kept in independent pens until weaning at 45d of age. After weaning all lambs were placed together on the same pasture for fattening for 4 months. Blood samples were taken at 24h after birth, at weaning and at the end of the fattening period (23 weeks). Results showed that no failure in the passive immune transfer was detected across treatments. Although artificially reared lambs at weaning had lower plasma levels of β-hydroxy-butyrate (-62%), HDL (-13%) and amylase (-25%) and higher levels of LDL (+38%) and alkaline phosphatase (+30%), these differences disappeared during the fattening period. Only the greater levels of calcium and the lower levels of haemoglobin and white blood cells detected at weaning in artificially reared lambs (+7.2%, -2.8% and -17.8%) persisted by the end of the fattening period (+4.3%, -3.3% and -9.5%, respectively). Minor diarrheal events from weeks 2 to 5 were recorded with artificial rearing, leading to lower growth rates during the first month. However, these artificially reared lambs caught up towards the end of the milk feeding period and reached similar weaning weights to NN lambs. During the fattening period NN lambs had a greater growth rate (+16%) possibly as a result of their greater early rumen development which allowed a higher feed digestibility during the fattening period in

comparison to NA lambs (+5.9%). As a result, NN lambs had heavier final body weights (+7.0%), but tended to have lower dressing percentage (-5.7%) than artificially reared lambs, thus no differences were noted in either carcass weight or in carcass conformation across treatments. In conclusion, the use of a colostrum alternative and milk replacer facilitated the successful rearing of lambs, reaching similar productive parameters; however special care must be taken to maximize the rumen development before weaning.

Keywords: animal performance, colostrum, health, milk replacer, weaning

Implications

This study revealed that artificial rearing of lambs with colostrum alternative and milk replacer represents an appropriate strategy to maximize the number of lambs weaned per ewe with a similar final BW achieved to lambs reared on the ewe. However, direct contact with the ewe provided a competitive advantage in naturally reared lambs allowing them to better develop their immune system and rumen function which led to increased BW gain during the fattening period.

Introduction

Two main systems exist for rearing offspring in ruminant production: in commercial dairy systems, or when dam milk is not available in sufficient amount or sanitary condition, newborns are separated from their dams within the first hours after birth and fed either milk replacer or whole milk; in contrast, in meat production systems, newborn animals generally remain with their dams until weaning. A recent study has reported that kid goats reared with their dams had greater rumen development than their twins that were fed on milk replacer and isolated from adult animals, despite both groups having access to the same solid feed (Abecia *et al.*, 2014). However, it remains unknown whether these differences are transitory or if they persist later in life during the fattening period.

Lambs are born hypogammaglobulemic due to the complexity of the synepitheliochorial ruminant placenta, which does not allow sufficient transfer of immunoglobulins from the dam to the fetus (Hernández-Castellano *et al.*, 2014, Hernández-Castellano *et al.*, 2015), thus IgG transfer from colostrum is vital for the neonatal health (Arguello *et al.*, 2004). Insufficient neonatal absorption of colostral immunoglobulins within the first day of life has been associated with failure of passive immunity transfer which is indicated when serum IgG levels are below a certain threshold (generally 10 mg/ml in calves, 12

74 mg/ml in goats and 15 mg/ml in lambs) leading to increased risk for neonatal diseases,
75 mortality and with a negative effect on adult health, longevity and performance (DeNise
76 *et al.*, 1989, Arguello *et al.*, 2004, Faber *et al.*, 2005, Alves *et al.*, 2015). As a result,
77 higher morbidity and mortality rates have been observed in colostrum-deprived lambs
78 (80 and 67%) than colostrum fed lambs (20 and 13%) (Hodgson *et al.*, 1992). In
79 addition, there is increasing evidence showing that nutritional management in the pre-
80 weaning period determines to a great extent the potential for milk production during
81 subsequent lactations: several studies have indicated that those heifers fed with a
82 greater volume of the same high quality colostrum (Faber *et al.*, 2005) and those with a
83 greater plasma concentration of IgG shortly after birth (DeNise *et al.*, 1989) had higher
84 milk yield than their counterpart control animals during their productive life. Moreover it
85 has been noted that increased growth rate before weaning results in positive effects on
86 milk yield in cattle (Soberon *et al.*, 2012). Thus, the general recommendation is to
87 actively feed lambs with colostrum from a freshly lambed ewe in order to maximize
88 passive immunity transfer. However, when ewe colostrum is scarce the
89 supplementation of lambs with colostrum alternatives may represent a strategy to
90 maximize the number of lambs weaned. Nevertheless, it remains unknown whether
91 these early life interventions in lambs could have similar long-lasting consequences to
92 those described in cattle.
93 In this study we hypothesized that nutritional interventions early in the life of the lambs
94 could have immediate effects on the animal´s health and performance, with some
95 effects persistent later in life under conventional production systems. These nutritional
96 interventions during the pre-weaning period consisted of 1) lambs remained with the
97 ewe (natural rearing) (NN), 2) ewe colostrum followed by artificial rearing with milk
98 replacer (NA), and 3) colostrum alternative supplementation and artificial rearing (AA).

Material and methods

Animals and diets

All animal procedures were carried out according to the Home Office Scientific Procedures, Act 1986 (PLL 40/3653; PIL 40/9798). Triplet lambs were used to provide similar genetic background, gestation environment and ewe colostrum in order to minimize the inter-animal variation across treatments. Thus, after pregnancy scanning, twenty-four pregnant Aberdale ewes carrying triplets were selected from the Aberystwyth University commercial flock. A total of 72 Aberdale-texel crossbreed lambs were born within an 8-day period (14th to the 22nd April). At birth umbilical cords were disinfected with iodine and lambs were weighed. One animal of each triplet set was randomly allocated to 1 of 3 experimental treatments. During this allocation process sex and initial body weight of the lambs was considered resulting in similar sex distribution (average 13 males and 11 females per group) and birth weights (3.8±0.8 kg) across treatments. All three lambs were kept with their mother in an individual pen during the first 24h after birth. Two lambs (NN and NA) were encouraged to suckle ewe colostrum by connecting them to a ewe´s teat four times over the first 24h (1, 2, 4 and 6 h after birth) until the gut filling was evident in order to ensure a high colostrum intake. Then, one of those lambs (NN) remained with its mother suckling ewe milk from birth to weaning, while the second lamb (NA) was separated from its dam after 24h and artificially reared with milk replacer. On the contrary, the third lamb (AA) was not encouraged to suckle ewe colostrum, instead it was immediately fed with 50g of colostrum alternative divided in two equal doses at 1h and 6h after birth followed by artificial rearing with milk replacer. In this latter group, no obvious signs of gut filling with ewe colostrum were noted suggesting a minimal intake of ewe colostrum. Colostrum

alternative was freshly prepared by mixing 25g of product (Lamb Volostrum, Volac Ltd.) in 50ml of water at 30ºC and provided by a stomach tube at each time (1h and 6h after birth). Milk replacer was prepared by mixing 200g of milk powder (Lamlac Instant, Volac Ltd.) with water to make up 1 litre of reconstituted milk following the manufacturer instructions. During their first week of life all lambs had access to heat lamps and warm milk replacer (39ºC) offered *ad libitum* using temperature controlled feeders (Ewe 2 Feeder, Volac Ltd, UK). Lambs that did not suckle were stomach tubed and trained to suck from a teat connected to the milk feeder. After one week of age all lambs were able to suckle and milk replacer was offered *ad libitum* at room temperature (average 12ºC) using two buckets connected to four teats for each experimental group. These milk buckets were emptied twice a day and thoroughly cleaned and rinsed, using soap and hot water.

At 24h after birth, blood was sampled (see below), and all animals were tagged and intramuscularly injected with 1 ml of AD_3E (NAPHA Veterinary, UK) to prevent vitamin deficiency. Then, all lambs from the same treatment were placed together in a single pen (10m×12m) with clean and dry barley straw bedding and *ad libitum* access to creep feed (NuGro CCF, UK), ryegrass hay and water (chemical composition described in Supplementary Table S1). During the milk feeding stage all three groups of animals were physically separated from each other (by approximately 1 m) but kept in the same building (average temperature of 12ºC, relative humidity of 86%, and an average of 10 hours of day light). Treatments NA and AA also had free access to milk replacer which was freshly prepared twice a day at 09:00h and 17:00h. Lambs from treatment NN shared a pen with their mothers that were fed twice a day with the same ryegrass hay and commercial concentrate (Wynnstay, High Production Ewes, UK). Ewes were physically separated from the NN lambs for 10 minutes during the concentrate feeding.

Group intakes of milk replacer and creep feed were recorded daily until weaning. Animals were inspected daily for signs of disease. The incidence of diarrheal events was recorded, and the severity was assessed based on a score from 1 (absence) to 4 (severe). Animals with a score above 3 received an intramuscular antibiotic treatment (Pen-Strep, Norbrook, UK). Lambs were weekly weighed using a digital balance to determine their growth during the entire duration of the experiment.

Animals were weaned at 45d of age by abrupt weaning and kept in the same building with the same solid feed for a further one week. When lambs were on average 8 weeks of age, all experimental lambs were grouped together on the same ryegrass pasture (*Lolium perenne*) with free access to creep feed until 10 weeks of age but not thereafter. Thus all lambs grazed the same pasture over 5 months (from June to November). When the average body weight (BW) of a given set of triplets reached the optimum slaughter weight (approximately 40kg and between 23 to 31 weeks of age), all three lambs were slaughtered in a commercial abattoir. Carcass weight and performance was assessed at an official abattoir according to the EUROP classification (Johansen *et al.*, 2006).

Sampling and analyses

Blood samples (5ml) were collected from the jugular vein at 24h after birth, at weaning (45d) and at the end of the fattening period (23wks). One blood subsample (2ml) was placed in a tube with anticoagulant (K_3-EDTA) mixed by inversion 10 times, kept at 4ºC and immediately analysed for haematology using a Mythic 18 Vet Haematology Analyser (Woodley Equipment Company Ltd., UK). This analysis determined levels of the main blood cells and their morphotypes (see below). A second subsample (3ml) was placed in a tube without anticoagulant; serum was harvested by centrifugation at

2,000×*g* for 15min and stored at -20ºC until analysis. Serum metabolites were determined using RX Daytona^{+} equipment (Randox Laboratories Ltd. UK).

Colostrum (10ml) and milk (50ml) samples were obtained by hand milking from each ewe at 24h after the birth of the first lamb and at 45d post-partum, respectively. Samples were kept frozen and milk and colostrum composition (Table 1) was determined using a milk analyser (LactoScope Advance FTIR, Delta Instruments, Netherlands). Concentration of IgG in serum and colostrum was determined using the Sheep IgG ELISA 96 well plate kit (Gen Way, USA, reference GWB-OVI374) after dilution (4×10^{-4} and 4×10^{-6} for serum and colostrum respectively) and absorbance at 450nm was measured using a plate reader (PowerWave XS2, BioTek, UK). Temperature corrected density (nD_{TC}) in serum samples (100µL) was measured in triplicate using an automatic digital refractometer (Reichert AR200 Ver 1.8, Ametek, Germany) and concentration of IgG in was estimated based on the regression equations described by Morril (2011): IgG (mg/ml) = $5919.1 \times nD_{TC} - 7946.1$

(Table 1 here)

Faecal analysis

At 23wks of age faecal grab samples were collected from each animal on two non-consecutive days, frozen and pooled by animal (30g DM approximately). On the same days as faecal sampling, ryegrass pasture was cut to 5 cm above soil level from 4 different locations of the field and immediately frozen for further analysis. The effect of the experimental treatments on pasture digestibility was estimated using the acid insoluble ash as an internal marker (Thonney *et al.*, 1979). For feed and faeces analyses, dry matter (DM) content was determined by drying in an oven at 105ºC for 24h. Organic matter (OM) concentration was determined by heating at 550ºC for 6h in a

muffle furnace. Nitrogen and carbon concentration was measured by the Dumas combustion method (Elementar analyser, Vario MAX cube, Germany). Neutral-detergent (NDF) and acid-detergent fibre (ADF) were determined using an Automated Fiber Analyzer (ANKOM 2000, USA) using heat stable amylase and sodium sulphide.

For faecal fingerprint analysis, samples were analysed as previously reported (Belanche *et al.*, 2017). Briefly, freeze dry samples were ground to a fine powder (IKA Analytical Mill, Staufer, Germany) and analysed by attenuated total reflectance (ATR) from 4000 to 600cm^{-1} using an Equinox 55 Fourier Transformed Infrared Spectrophotometer (Bruker Ltd, Coventry, UK)), and scanned using the Golden Gate ATR accessory (Specac Ltd., Slough, UK). Infrared settings and data collection were conducted as previously reported (Belanche *et al.*, 2014). Fourier transformed infra-red (FTIR) spectra were imported into Matlab (version 2007b, The MathWorks Inc., Natick, USA), averaged, transformed to the first Savitsky-Golay derivative to smooth baseline noise and improve spectral resolution using a 13-point window, and then mean centre normalized (mean=1, Standard Deviation=1). Data were then analysed by non-parametric permutational multivariate analysis of variance using PRIMER-6 software (PRIMER-E Ltd., Plymouth, UK). Statistical signification was calculated after 999 random permutations of residuals under a reduced model using the Monte Carlo test. For graphical interpretation, principal component analysis was conducted and a Canonical variate analysis was performed based on the data compiled in the main principal components (Genstat 18th Edition, VSN International, Hemel Hempstead, UK).

Calculations and statistical analysis

Haematological analysis determined the levels of red blood cells, haemoblobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH),

mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RBCDW), white blood cells and its morphotype percentages, platelets, mean platelets volume (MPV), thrombocrit and platelet distribution width (PDW). While the plasma metabolic analysis measured: calcium, glucose, β-hydroxybutyrate (BHB), cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), albumin, creatinine, urea, ammonia, L-lactate dehydrogenase and alkaline phosphatase levels. Globulins and LDL concentrations in plasma were mathematically calculated:

Globulins = Total proteins – Albumin

LDL = Cholesterol – HDL – (Triglycerides / 5)

To evaluate the effect of experimental treatments on blood parameters, data were analysed using an ANOVA as follows:

$$Y_{ijk} = \mu + R_i + T_j + RT_{ij} + T_k + A_l + e_{ijkl}$$

where Y_{ijk} is the dependent, continuous variable (n = 24), μ is the overall mean, M_i is the fixed effect of the type of rearing (i = NN vs NA vs AA), T_j is the fixed effect of the animal age (j = weaning vs fattening), FV_{ij} is their interaction, S_k is the random effect of the triplet set used as a block (k = 1 to 24), A_l is the random effect of the animal (j = 1 to 72) and e_{ijkl} is the residual error. For animal weight, growth and carcass performance data, the term sex (male vs females) was also included as a fixed effect. When significant effects were detected across treatments, means were compared by Fisher's protected LSD-test (Genstat 18th Edition, VSN International, Hemel Hempstead, UK). Significant effects were declared at P<0.05.

Results

Animal health

At 24h after birth all animals remained in good health and neither haematological nor plasma lgG differences were observed across treatments (Table 2). Animals artificially reared (NA and AA) suffered a greater incidence of diarrhoea episodes than NN lambs from 2 to 5 weeks of age (P=0.001) but this effect disappeared thereafter. Antibiotic usage was also higher for NA and AA lambs than for NN lambs (P<0.001) and the number of animals with recurrent diarrhoea which required more than 2 antibiotic doses were 0, 6 and 9 for NN, NA and AA lambs, respectively.

(Table 2 here)

The age of the lambs exerted a major effect on the blood cell distribution (Table 3) and the concentration of most plasma metabolites (Table 4). At weaning animals had a greater concentration of red blood cells, haemoglobin, RBCDW, lymphocytes, platelets, thrombocrit and plasma levels of calcium, glucose, cholesterol, triglycerides, HDL, LDL, albumin, creatinine, amylase and alkaline phosphatase than animals at fattening (P<0.001). On the contrary, at fattening animals had a greater concentration of white blood cells, monocytes, granulocytes, MPV, PDW and plasma levels of BHB, total proteins, globulins and urea (P<0.001). However, artificial rearing also had a small mid- and long-term effect on the animals´ health (Table 3). NN lambs had a greater haemoglobin (P=0.012), haematocrit (P=0.044), white blood cells (P=0.007) and calcium levels than NA and AA lambs, independently of the age considered. Moreover a significant interaction was observed for several metabolites and haematological parameters: at weaning NN lambs had greater RBCDW (P>0.001), BHB (P<0.001), HDL (P=0.001) and amylase plasma levels (P<0.001), as well as lower MCHC (P=0.014), PDW (P=0.004), LDL (P=0.009) and alkaline phosphatase (P<0.001) were observed in NN lambs than in NA or AA but no such differences were observed at fattening.

(Table 3 and Table 4 here)

Animal performance

Average group intake of milk replacer remained constant until week 3 (300 g DM/d per lamb) and linearly increased thereafter reaching 550 g DM/d at weaning for AA and NA groups while milk intake in NN lambs was not recorded. Group intake of creep feed also remained low and constant until week 4 across treatments, and increased linearly thereafter reaching an average of 256, 137 and 96 g DM/d at weaning for treatments NN, NA and AA, respectively. No differences in body weight (BW) were observed at birth across treatments, but NN lambs had a greater BW than NA and AA lambs from week 2 to 5, these differences disappeared during the weaning stage and reappeared from week 11 onwards (Figure 1). No differences in the average daily gain (ADG) were observed before weaning (Table 5), but NN lambs had a greater ADG during the fattening period calculated from weaning to 23 weeks of age (P<0.001). In terms of carcass composition, NN lambs had a higher slaughter weight than NA and AA lambs (P<0.001), but NN lambs tend to have a lower dressing percentage resulting in similar carcass weight and conformation across treatments. Male lambs tended to have a greater BW at birth and ADG during the fattening period (P=0.047) however no differences were observed in carcass conformation.

(Figure 1 and Table 5 here)

Pasture utilization

The chemical structure of the faecal samples tended to differ (P=0.079) between treatments based on the PERMANOVA analysis of the FTIR spectral data

297 (Supplemental Table 2). Canonical variate analysis (Figure 2) compiling the information
298 of the first 15 principal components (representing 98.1% of the total variance) showed
299 that these differences were more obvious between NA and the other two experimental
300 groups. In terms of pasture digestibility (Table 6), values were always highest for NN
301 lambs: NN and AA lambs had higher digestibility for DM (P<0.001), C (P<0.001) and N
302 (P=0.003) than NA lambs, while no differences were observed in NDF and ADF
303 digestibility.
304 (Figure 2 and Table 5 and Table 6 here)
305
306 **Discussion**
307 *Effect of colostrum alternative*
308 Colostrum products have been shown to provide a degree of passive immunity transfer
309 (Seymour *et al.*, 1995, Castro *et al.*, 2007), although the results vary greatly depending
310 on the product used, colostrum preservation methods, dosage techniques and inter
311 animal variation (Arguello *et al.*, 2004). As a result, colostrum products that typically
312 contain lacteal-derived or plasma-derived IgG are classified as either colostrum
313 replacers or colostrum supplements depending on their ability to raise serum IgG
314 concentration above a certain threshold (typically 15 mg/ml in lambs) (Alves *et al.*,
315 2015). Colostrum supplements (as in our study) can be used to increase the amount of
316 IgG fed to lambs when only low or medium quality / quantity colostrum is available.
317 However, supplements cannot replace high quality colostrum which is still considered
318 the gold standard for feeding newborn lambs (Jones *et al.*, 2004). Our study aimed to
319 simulate two real scenarios in the artificial rearing of lambs: one (NN and NA lambs)
320 consisting of maximizing colostrum intake by encouraging lambs to suckle for at least 4
321 times from the ewe; and an alternative strategy (AA lambs) based on colostrum

alternative supplementation of lambs with an insufficient intake of ewe colostrum. To achieve this situation, AA lambs were not encouraged to suckle and had to compete with their two siblings for the remaining ewe colostrum. A rapid change in the colostrum composition to transitional milk has been described during the post-partum period (Alves *et al.*, 2015). In our study, despite the late sampling of ewe colostrum (24h after the first lamb was born), the IgG concentrations (average 42.2 g/l) were comparable to published literature (from 15.7 to 65 g/l) in which the samples were collected just after parturition (Vatankhah, 2013, Alves *et al.*, 2015, Hernández-Castellano *et al.*, 2015), possibly as a result of a higher colostrum production in high prolific ewes. As a result, only one lamb had an IgG concentration below 15 mg/ml at 24h after birth suggesting effective overall passive immunity transfer across treatments (Alves *et al.*, 2015). This may explain the lack of differences in terms of growth, haematology parameters and blood metabolites levels between NA and AA lambs, as well as, the absence of deaths before weaning. Moreover, the high level of easily digestible energy and protein in the colostrum alternative also seems to represent an important source of nutrients for the lambs during its first hours of life to maintain body temperature and good health (Jones *et al.*, 2004). Thus, the supply of colostrum alternative after birth can be considered an appropriate strategy to prevent health problems and maximize the number of lambs weaned per ewe when ewe colostrum is insufficient.

Effect of artificial rearing on lamb´s health

This study does not attempt a direct comparison of the effects of milk replacer vs maternal milk since artificial rearing involves the replacement of the contributions made by the ewe which are essential to the growth and development of the lamb. This not only includes the feed supply but also the warmth, shelter and “mothering” normally

provided by the ewe. Our experiment showed a greater incidence of diarrhoea events in artificially reared lambs than those reared on the ewe. These diarrhoea episodes appeared from week 2 to week 5; they were very mild (<2.0 scored) and required an average of 1.2 antibiotic doses per lamb, whilst antibiotic usage in NN lambs was negligible. Although these diarrheal events did not trigger any deaths, they could explain the lower ADG for NA and AA lambs during the first 5 weeks. Similar diarrhoea events starting at 2 weeks of age have been described in calves and various pathogens compatible with enteric infections have been identified in the necropsy (i.e. *Salmonella*, *Cryptosporidium parvum*, *Escherichia coli* and coronavirus) (Quigley *et al.*, 2006). Various studies have investigated the effect of different artificial milk feeding strategies to prevent diarrheal events and to improve animal performance: Jasper and Weary (2002) concluded that *ad libitum* nipple feeding of whole milk to dairy calves vs restricted can increase weight gain with no diarrheal problems nor detrimental effects on feed intake after weaning. While Quigley (2006) observed that calves fed a variable amount of milk replacer (peaking at 3 weeks of age with 908 g/d) had greater ADG but also increased incidence of diarrhoea that required added veterinary treatment in comparison to those fed a fixed amount (454 g/d). Thus, it seems that our artificial rearing strategy based on the *ad libitum* access to milk replacer might explain the incidence of moderate diarrhoea but did help to prevent feed competition between lambs, since lambs in contrast to calves tend to be reared in groups with a large number of animals. More research is needed to assess whether these diarrheal events could be minimized by using alternative rearing systems such as automatic feeding machines.

Although most lambs remained in good health from birth to slaughter, the haematological analysis revealed that NN lambs had higher levels of white blood cell at weaning in comparison to artificially reared lambs (+21.6%), and those differences

persisted during the fattening period (+10.5%). It has been shown that colostrum and milk have viable cells, including neutrophils and macrophages, which secrete a range of immune-related components (Stelwagen *et al.*, 2009). Our findings are in line with this observation and suggest that direct contact with adult animals in NN may also represent an important exposure to antigens which may help in the immune system development of young lambs with long-lasting effects on the levels of white blood cells. Moreover, artificially reared lambs had lower haemoglobin levels (-2.8%) and haematocrit (-5.3%) at weaning in comparison to NN lambs. The variation in the size of red cells (anisocytosis) provided an insight of the potential reasons of slight signs of anaemia. Since neither the size of the red blood cells (MCV) nor the amount of haemoglobin per cell (MCH) were affected, it seems that the normocytic anaemia was very mild and partially compensated by a greater amount of haemoglobin per unit of volume (MCHC +2.6%). Despite this lack of severity, artificially reared lambs still had lower levels of haemoglobin (-3.3%) and haematocrit (-0.4%) during the fattening period suggesting a small but long term effect of the type of rearing strategy on the animals health. On the contrary, NN lambs had a higher coefficient of variation in red blood cell distribution width (RBCDW, +20.0%) which is compatible with early stages of iron deficiency at weaning in animals having limited amounts of milk (Blaxter *et al.*, 1957), possibly as a result of a lower milk intake and lower iron content in the ewe milk in comparison to lambs fed milk replacer *ad libitum*. This observation was supported by the lower blood calcium concentration in NN lambs at weaning (-6.7%) and fattening (-4.2%). Increases in plasma glucose and urea concentrations have been associated with higher artificial milk intake in calves (Quigley *et al.*, 2006). However, in our study all experimental treatments had similar glucose, urea and total protein levels at weaning, possibly because a lower milk intake in NN lambs during late milk feeding period in comparison

to those fed milk replacer was compensated by a greater creep feed intake (256 vs 116 g/d). Our experiment indicates that protein and energy sources included in the milk replacer were highly digestible since no differences in the plasma concentration of metabolites related with the protein (total proteins, albumin, globulin, creatinine, urea and ammonia) and energy (glucose) metabolism were detected across treatments. These findings agree with the similar content of urea nitrogen, total protein, albumin and globulin in the serum of lambs fed milk replacers made up of milk protein or other protein sources (Huang *et al.*, 2015). Most of the milk bypasses the rumen through the oesophageal groove, thus high milk intake in artificially reared lambs may increase the amino acid flow to the small intestine leading to an increase in the deamination processes occurring in the liver as was reflected by increased levels of alkaline phosphatase (+30%) as an indicator of the liver stress (Reichling & Kaplan, 1988). On the contrary, solid feed (carbohydrates and proteins) is fermented in the rumen producing volatile fatty acids and ammonia as the main fermentation end product. Thus, the increased levels of β-hydroxy-butyrate in NN at weaning (+2.6-fold times) suggest a greater physiological and fermentative development of the rumen. Although cholesterol and triglyceride concentrations were unaffected by the experimental treatments, artificially reared lambs had lower levels of HDL (-13%) and higher levels of LDL (+38%) at weaning than NN lambs. Increased blood levels of LDL is considered a circulatory risk factor which is mainly determined by diet, physical activity, genetics, sex and age (Sigurdardottir *et al.*, 2002). Overall, our data also showed that most of the haematological and metabolite differences observed at weaning were transient and tended to disappear later in life with no further effects on the animal's health.

Effect of artificial rearing on productive performance

This study revealed that in comparison with artificially reared lambs, NN lambs had a higher neonatal growth suggesting that the ewe mothering instinct helps lambs to suckle more efficiently during the first days of life. Moreover this competitive advantage was maintained until 3 weeks after birth, when NN lambs reached the greatest differences in BW (+10.5%), corresponding with the peak in the lactation curve described for crossbred ewes rearing lambs (Cardellino & Benson, 2002). However, these differences tended to disappear as weaning approached , possibly due to the increased milk intake recorded for the artificially reared lambs (average 2.9 L/d), resulting in similar BW at weaning across treatments. This observation agrees with the lack of differences in weaning weights reported for Comisana lambs reared artificially or conventionally (Napolitano *et al.*, 2002).

However, differences in BW gain reappeared after weaning despite all lambs being grazed together on the same pasture. As a result, NN lambs had a greater growth during the fattening period (+16%) and higher BW from week 13 onwards. Several reasons could explain these findings: i) The greater solid feed intake observed in NN lambs at weaning (256 vs 116 g DM/d) has been described as a key factor which promotes the rumen physiological development in calves and facilitates a smooth transition to the solid diet (Khan *et al.*, 2011). ii) The direct contact with adult animals represents a source of microbes (i.e. bacteria, protozoa, methanogens, anaerobic fungi) which are crucial for the development of the symbiotic rumen microbiota (Belanche *et al.*, 2010, Belanche *et al.*, 2011). iii) Adult animals teach young animals in terms of feeding behaviour since the presence of adult companions has been reported to increase solid feed intake and performance of calves before and after weaning (Vieira *et al.*, 2012) as was noted in our experiment.

Our findings also suggest that the greater BW gain in NN lambs during the fattening period may in part be explained by greater feed DM digestibility (+5.9%) in comparison to NA lambs, although differences were less obvious (+2.0%) when compared with AA lambs. These differences in forage utilization were also observed based on the fingerprint analysis of faecal samples using FTIR spectroscopy. As a result, NN lambs reached a greater final BW (+7.0%) at slaughter but they performed substantially worse in dressing percentage (-5.7%) leading to similar carcass weight, carcass confirmation and fatness. This observation indicates that NN lambs may have a greater rumen size, slower rumen transit time or greater wool yield all of which could reduce the killing out percentage. These findings support previous observations which suggest that rearing lambs on the ewe, and the early intake of solid feed are important drivers not only for the rumen anatomical enlargement, but also for the physiological and microbiological development (Yáñez-Ruiz *et al.*, 2015). Thus, more research is needed based on a better description of the rumen dynamics of feed utilization, rumen microbiota and animal behavioural studies to elucidate which factor plays a greater role on animal resilience and productivity during the post-weaning processes as well as later in life.

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559 fermentation through interventions during early life: a review. *Frontiers in microbiology* 6, 1-
560 12

561 **Table 1.** Colostrum and milk composition

	Colostrum		Milk	
	Natural	Alternative[1]	Natural	Replacer[2]
CP, %	22.6	22.1	4.35	4.77
Fat, %	15.5	4.52	5.51	4.84
Lactose, %	2.79	2.82	4.90	7.33
Solids, %	40.9	30.5	15.4	17.4
Solids non-fat, %	27.2	27.9	10.4	13.1
IgG, g/l	42.2	32.1		

562 [1]Values after mixing 25g of colostrum alternative (Lamb Volostrum, Volac Ltd.) with 50
563 ml of water

564 [2]After mixing 200g of milk replacer (Lamlac Instant, Volac Ltd.) with water to make up 1
565 lire of reconstituted milk

566 **Table 2**. Effect of colostrum alternative and artificial rearing on plasma IgG levels and
567 haematology at 24h after birth and incidence of diarrhoea before weaning.

Type of rearing	NN	NA	AA	SED[1]	*P*-value
Red blood cells (10^6/µl)	8.20	7.77	8.01	0.221	0.152
White blood cells (10^3/µl)	6.48	5.82	6.01	0.539	0.461
Platelets (10^3/µl)	630[a]	502[b]	575[ab]	48.8	0.041
Haematocrit (%)	38.0	36.2	37.3	1.14	0.276
ELISA IgG (mg/ml)	40.1	45.6	37.1	4.19	0.135
Refractometer IgG (mg/ml)	38.3	38.3	32.5	2.88	0.075
Diarrhoea score[2]					
Week 2	1.13[b]	1.83[a]	2.04[a]	0.229	<0.001
Week 3	1.29[b]	1.96[a]	2.33[a]	0.269	0.001
Week 4	1.08[b]	1.96[a]	1.92[a]	0.252	0.001
Week 5	1.04[b]	1.58[a]	1.96[a]	0.227	<0.001
Week 6	1.04	1.08	1.25	0.121	0.201
Week 7	1.04	1.04	1.17	0.108	0.415
Antibiotic usage (doses/lamb)[3]	0.08[b]	0.96[a]	1.42[a]	0.333	<0.001

568 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum
569 alternative and artificial milk feeding.
570 [1]Standard error of the difference among means. Within a row means without a common
571 superscript differ ($P<0.05$).
572 [2]Diarrhoea score: 1 absence, 2 very mild, 3 moderate and 4 severe.
573 [3]Intramuscular Penicillin-Streptomycin

574 **Table 3.** Effect of colostrum alternative and artificial rearing on haematology and blood metabolites in lambs.

	Weaning (45 days)			Fattening (23 wks)				*P*-value		
Type of rearing[1]	NN	NA	AA	NN	NA	AA	SED[1]	Rearing	Age	R×A
Red blood cells (10^6/µl)	11.4	11.4	11.7	11.3	10.9	11.1	0.237	0.148	<0.001	0.178
Haemoglobin (g/dl)	11.5	11.0	11.3	11.1	10.6	10.8	0.236	0.012	<0.001	0.940
Haematocrit (%)	38.3	35.8	36.7	36.2	34.6	37.5	1.422	0.044	0.210	0.320
MCV (fL)	33.6	31.5	32.1	32.1	31.9	34.0	1.435	0.384	0.799	0.240
MCH, (pg)	10.1	9.72	9.77	9.83	9.74	9.78	0.201	0.397	0.472	0.415
MCHC (%)	30.0^{b}	30.8^{a}	30.8^{a}	30.6^{a}	30.6^{a}	30.7^{a}	0.208	0.005	0.374	0.014
RBCDW (%)	25.4^{a}	20.0^{b}	20.7^{b}	17.9^{c}	18.2^{c}	17.9^{c}	0.508	<0.001	<0.001	<0.001
White blood cells (10^3/µl)	7.95	6.31	6.76	8.91	8.40	7.73	0.557	0.007	<0.001	0.369
Lymphocytes (%)	56.5	56.9	54.4	53.2	47.6	51.1	2.365	0.38	<0.001	0.121
Monocytes (%)	11.6	11.9	10.9	13.7	14.7	14.4	0.621	0.349	<0.001	0.149
Granulocytes (%)	31.9	31.2	34.7	33.1	37.6	34.5	2.091	0.432	0.023	0.057
Platelets (10^3/µl)	1982^{a}	1419^{b}	1695^{ab}	548^{c}	616^{c}	639^{c}	179.0	0.145	<0.001	0.055
MPV (fl)	5.20	4.90	4.71	5.71	5.98	5.75	0.364	0.641	<0.001	0.400
Thrombocrit	1.10	0.72	0.82	0.29	0.33	0.62	0.189	0.301	<0.001	0.078
PDW (%)	30.1^{d}	36.0^{c}	34.9^{c}	46.0^{a}	42.1^{b}	44.0^{ab}	2.453	0.694	<0.001	0.004

575 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding.

576 MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration;

577 RBCDW, red blood cell distribution width; MPV, mean platelets volume; PDW, platelet distribution width

578 [1]Standard error of the difference among means. Within a row means without a common superscript differ (P<0.05).

579 **Table 4.** Effect of colostrum alternative and artificial rearing on blood metabolites in lambs.

	Weaning (45 days)			Fattening (23 wks)				*P*-value		
Item[1]	NN	NA	AA	NN	NA	AA	SED[1]	Rearing	Age	R×A
Calcium (mM)	2.33	2.52	2.48	1.84	1.92	1.93	0.116	0.075	<0.001	0.854
Energy										
Glucose (mM)	5.47	5.89	5.75	3.50	3.56	3.56	0.306	0.277	<0.001	0.881
BHB (µM)	265[b]	100[c]	100[c]	342[a]	372[a]	394[a]	29.57	0.006	<0.001	<0.001
Lipids (mM)										
Cholesterol	2.82	2.91	2.79	1.27	1.31	1.23	0.184	0.596	<0.001	0.822
Triglycerides[2]	0.78	0.72	0.75	0.24	0.21	0.24	0.061	0.481	<0.001	0.885
HDL	1.91[a]	1.65[b]	1.66[b]	0.62[c]	0.65[c]	0.61[c]	0.104	0.489	<0.001	0.001
LDL[3]	0.76[b]	1.11[a]	0.98[a]	0.60[c]	0.61[c]	0.57[c]	0.098	0.061	<0.001	0.009
Proteins, (g/l)										
Total Proteins	45.4	46.7	46.3	65.3	67.3	66.6	2.534	0.619	<0.001	0.984
Albumin	32.9	33.5	33.3	30.2	31.1	31.2	1.019	0.551	<0.001	0.952
Globulin[2]	12.5	13.3	13.0	35.1	36.2	35.4	1.730	0.732	<0.001	0.994
Creatinine (µM	83.0	85.8	87.9	78.3	80.4	78.5	4.640	0.583	0.051	0.656
Urea, mM	3.85	3.95	3.82	9.98	9.85	10.1	0.342	0.933	<0.001	0.803
Ammonia (µM)	83.6	81.9	85.2	84.9	86.0	89.7	5.690	0.505	0.548	0.747
Enzymes (U/l)										
Amylase	25.7[a]	20.3[b]	18.3[b]	12.5[c]	10.8[c]	12.6[c]	1.721	0.037	<0.001	<0.001
L-lactate dehydrogenase	1171	1238	1112	1163	1093	1098	64.40	0.178	0.262	0.501
Alkaline Phosphatase	637[b]	841[a]	819[a]	177[c]	184[c]	183[c]	44.00	0.005	<0.001	<0.001

580 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding.

581 [1]Standard error of the difference among means. Within a row means without a common superscript differ (P<0.05).

582 [2]BHB, beta-hydroxybutyrate; HDL, high desity lipoproteins; LDL, low density lipoproteins.

583 [3]Mathematically calculated: LDL= Cholesterol – HDL – (Triglycerides / 5); Globulin = Total Proteins – Albumin

584 **Table 5.** Effect of colostrum alternative and artificial rearing on animal and carcass performances in lambs.

	Type of lactation			Sex			P-value	
Item[2]	NN	NA	AA	Males	Females	SED[1]	Rearing	Sex
Animal performance								
BW at birth (kg)	3.81	3.89	3.88	4.07	3.56	0.124	0.794	0.005
BW at weaning 45d (kg)	18.5	18.9	18.3	19.1	18.0	0.572	0.583	0.001
BW at fattening, 23 weeks (kg)	38.6[a]	37.2[b]	35.3[b]	38.7	35.2	1.022	0.004	0.035
ADG from 0 to 45 days (g/d)	325	332	318	332	319	5.110	0.568	0.444
ADG from 45d to 23 weeks (g/d)	176[a]	153[b]	150[b]	170	150	5.050	<0.001	0.047
Carcass performance								
Final BW (kg)	42.3[a]	40.4[b]	38.7[b]	41.4	39.5	0.754	<0.001	0.155
Warm carcass weight (kg)	18.3	18.2	17.6	18.6	17.4	0.532	0.624	0.490
Dressing percentage (%)	43.1[b]	45.3[a]	46.2[a]	45.3	44.2	1.390	0.052	0.311
Conformation[3]	3.78	3.63	3.61	3.82	3.52	0.167	0.750	0.853
Fatness[3]	2.72	2.74	2.76	2.76	2.76	0.166	0.971	0.495

585 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding.

586 [1]Standard error of the difference among means. Within a row means without a common superscript differ (P<0.05).

587 [2]BW, body weight; ADG, average daily gain.

588 [3]EUROP classification: Conformation. E=5. U=4. R=3. O=2. P=1. Fatness: 1=1. 2=2. 3L=3. 3H=3.5. 4L=4. 4H=4.5. 5=5.

589 **Table 6**. Effect of artificial rearing on total tract digestibility (% in DM basis) in grazing
590 lambs (23 weeks of age).

Item[2]	NN	NA	AA	SED[1]	P-value
DM	66.3[a]	62.6[b]	65.0[a]	0.83	<0.001
C	61.7[a]	56.8[b]	60.3[a]	1.02	<0.001
N	75.5[a]	73.2[b]	75.1[a]	0.69	0.003
NDF	51.7	50.7	53.8	1.36	0.143
ADF	38.4	34.2	36.5	2.47	0.327

591 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum
592 alternative and artificial milk feeding.
593 [1]Standard error of the difference among means. Within a row means without a common
594 superscript differ (P<0.05).
595 [2]DM, dry matter; C, carbon, N, nitrogen, NDF, neutral detergent fibre; ADF, acid
596 detergent fibre.

Figure 1. Effect of colostrum alternative and artificial rearing on lamb´s growth. NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding. Standard error of the mean level of signification is depicted: ns, not significant, * P<0.05, ** P<0.01

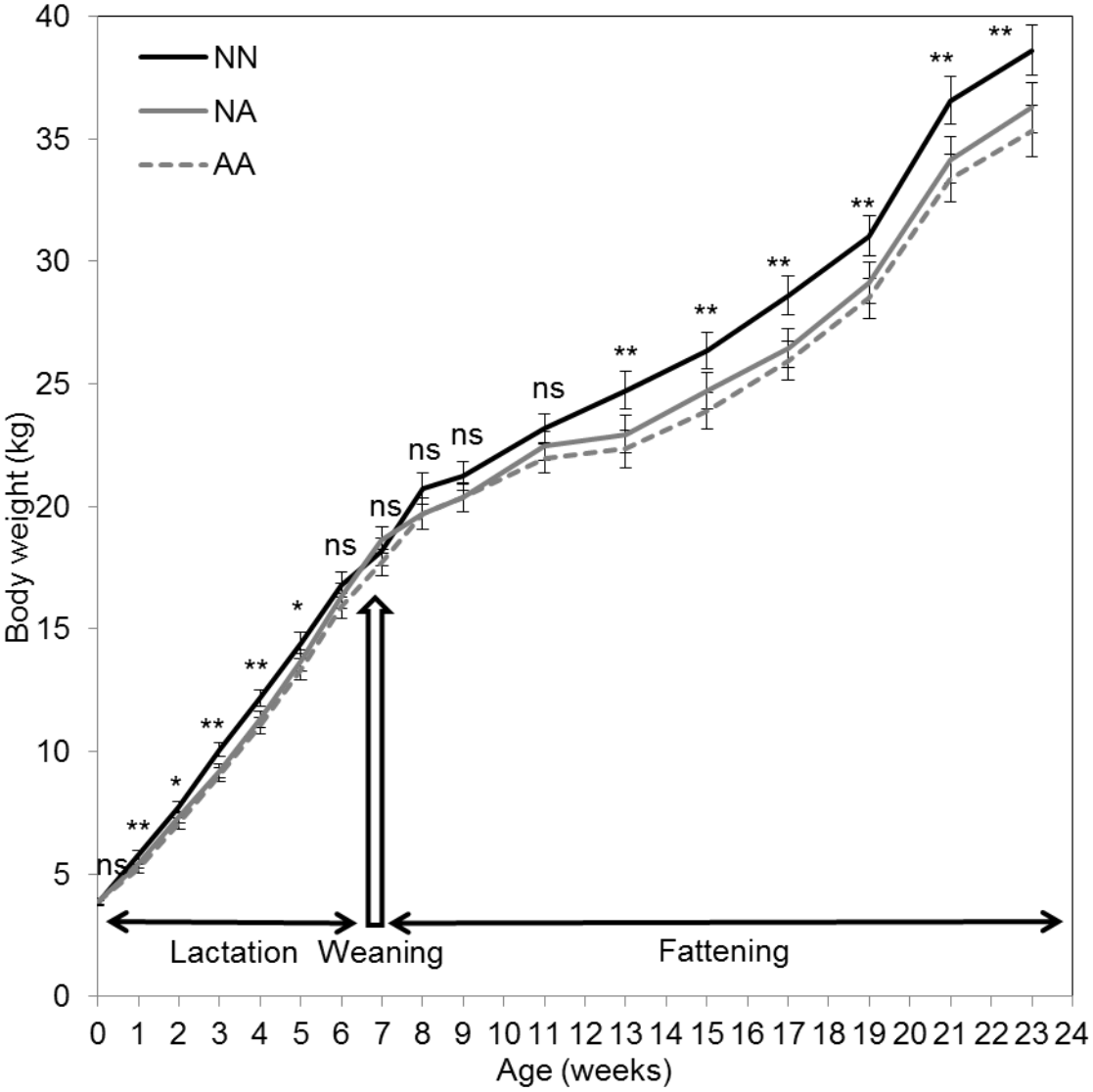
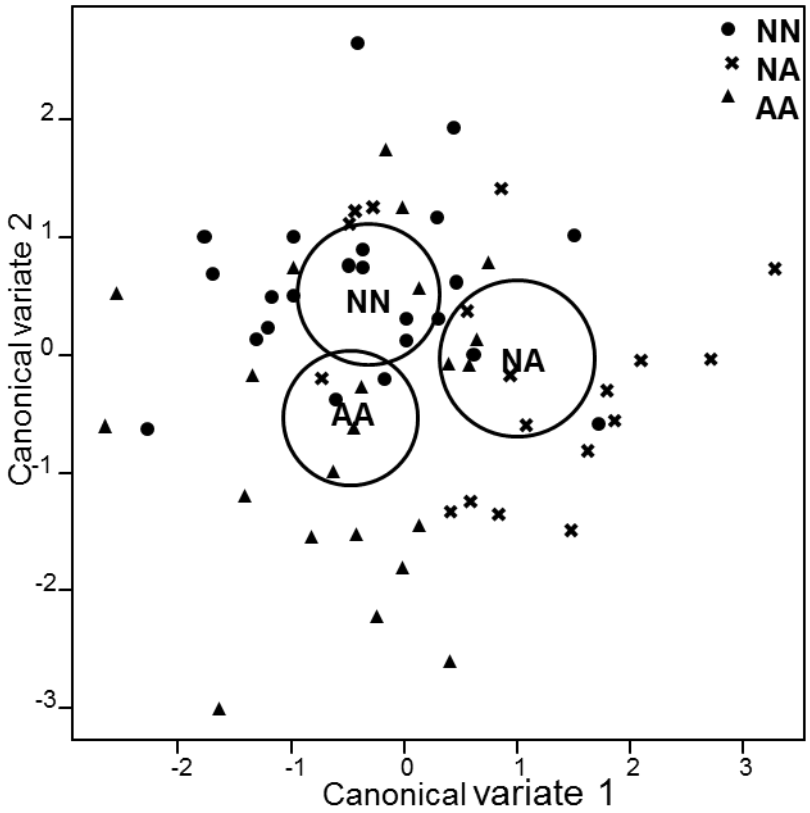


Figure 2. Canonical variate analysis illustrating the impact of nutritional intervention in early life on the faecal FTIR spectra from lambs of 23 weeks of age. NN, natural rearing (circles); NA, ewe colostrum and artificial milk feeding (crosses); AA, colostrum alternative and artificial milk feeding (triangles). Big circles indicate the 99% confidential interval of the mean for each treatment.



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619

Short- and long-term effects of different colostrum and milk feeding strategies on health and performance in growing lambs

Alejandro Belanche, Jessica Cooke, Eleanor Jones, Hilary J. Worgan and Charles J. Newbold

Table S1. Chemical composition (g/kg DM) of the main experimental feeds.

Items[1]	Creep-feed	Hay	Pasture
OM	926	936	904
CP	183	61	114
NDF	528	644	510
ADF	139	346	221
C/N ratio	15.0	45.4	24.0

[1]DM, dry matter; CP, crude protein, NDF, neutral detergent fibre; ADF, acid detergent fibre; C/N ratio, carbon to nitrogen ratio.

631 **Table S2.** PERMANOVA illustrating the effect of colostrum alternative and artificial

632 lactation on the faecal FTIR spectra in grazing lambs (23 weeks old).

Type of lactation[1]	Pseudo-F	P-value
Treatment effect	1.79	0.079
Pair-wise comparisons		
NN vs NA	1.39	0.135
NN vs AA	1.31	0.130
NA vs AA	1.56	0.099

633 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum

634 alternative and artificial milk feeding. Greater Psuedo-F and lower P-values indicates

635 differences between treatments.

636

637